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Proton-potassium exchange during fermentation of glucose and glycerol by *Escherichia coli* hydrogenase mutant at alkaline and acidic pH

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E. coli possesses four hydrogenses, which responsible for molecular hydrogen (H_2) formation and oxidation. It was demonstrated that *E. coli* during fermentation of glucose depending of medium pH H_2 produces via two forms of FHL-1 and FHL-2, constituted by formate dehydrogenase H and hydrogenase 3 (H3) or hydrogenase 4 (H4): at alkaline pH FHL-2 was shown to relate with the proton translocating F_0F_1-ATPase and potassium uptake TrkA system [1]. Relation of hydrogenases to F_0F_1-ATPase was also established during bacterial glycerol fermentation [2].

In this study was investigated proton-potassium exchange in *E. coli* hydrogenase new mutant Δ*hypF* with defective all four hydrogenses and wild type strain BW25113 pH 5.5 and pH 7.5 fermenting glucose and glycerol. During fermentation of glucose at alkaline and acidic pH was observed classical medium acidification and potassium ions uptake, which was inhibited by F_0F_1-ATPase inhibitor *N,N'*-dicyclohexylcarbodiimide (DCCD), pointing out the relation of this processes with proton translocating F_0F_1-ATPase. During fermentation of glucose at alkaline and acidic pH H^+ extrusion was suppressed 1.2 fold in Δ*hypF* mutant compared with wild type strain. K^+ uptake was suppressed 2 fold at alkaline pH, whereas at acidic pH it was stimulated 1.4 fold. During glycerol fermentation in *E. coli* wild type protons expelled via F_0F_1-ATPase with low rate (DCCD-sensitive fluxes are also observed) and potassium uptake was absent compared with the glucose fermenting cells at both pHs. While, H^+ extrusion was suppressed 1.7 fold at both alkaline and acidic pH in Δ*hypF* mutant compared with wild type strain fermenting glycerol.

The results indicate that during glycerol fermentation hydrogensaes participate in H^+ extrusion more than in glucose fermenting cells, moreover, during glucose fermentation depending of medium pH they have different relationship and role in potassium ions uptake possesses and systems.

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doi:10.1016/j.bbabio.2012.06.397

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Photophosphorylation Dependent CO_2 Fixation in Isolated Thylakoids of Cyanobacteria, A Case for Localized Proton Gradients

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Photophosphorylation is known to produce ATP using chemiosmotic potential generated across the thylakoid membranes that couples the photosynthetic electron transport to ATP-synthesis in photosynthetic bacteria and chloroplasts. The basic assumption of chemiosmotic hypothesis is that there should be enclosed vesicles for buildup of delocalized proton gradients. Although the chemiosmotic hypothesis and the role of such delocalized proton accumulation has been widely accepted in bioenergetics for ATP production, there are certain observations which suggest that protons constrained to localized domains rather than delocalized within vesicles would also be important in ATP production.

We have been interested in demonstrating association of Calvin cycle enzymes with thylakoid membranes in cyanobacterial model systems [1,2,3]. The thylakoids from cell free extracts of cyanobacteria, *Synechococcus* and *Synechocystis*, when fractionated by successive ultracentrifugation at 40,000×*g*, 90,000×*g* and 150,000×*g* yielded three distinct fractions designated as 40 k, 90 k and 150 k segments. The native thylakoid membranes appeared flat when analyzed by transmission electron microscopy [4] and showed no evidence for vesicles formation in biochemical assays. All these fractions showed presence of Calvin cycle enzymes and components of light reactions as well as that of Chloroplast ATP synthase. These isolated native thylakoids could carry out photophosphorylation dependent CO_2 fixation activity involving light dependent synthesis of ATP by ATP synthase and its subsequent use by Calvin cycle enzymes to fix $^{14}CO_2$. Interestingly, among the three fractions, the 150 k segments showed highest activity of photophosphorylation dependent CO_2 fixation suggesting that the supramolecular organization of components of light and dark reactions was optimum in these thylakoid segments. Photophosphorylation dependent CO_2 fixation was observed under conditions of cyclic as well as noncyclic photophosphorylation using methyl-viologen as acceptor and also in presence of uncoupler gramicidin.

These results suggested that the isolated thylakoid segments in 150 k fraction could generate ATP in absence of vesicles suggesting a role of localized proton gradient in this activity.

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doi:10.1016/j.bbabio.2012.06.398

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Computational investigation of O_H and O states in the catalytic mechanism of cytochrome *c* oxidase

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Cytochrome *c* oxidase (CcO) catalyzes the four electron reduction of molecular oxygen to water, and converts the free energy of the reaction to an electrochemical proton gradient by proton pumping across the mitochondrial or bacterial membrane [1]. During continuous turnover of the enzyme, two protons are pumped, and another two